from a single organism, stably inserting into the clones a substrate that is fluorescent in the presence of the activity of interest, screening the library with a fluorescent analyzer that detects bioactive fluorescence, and identifying clones detected as positive for bioactive fluorescence. Fluorescence is indicative of DNA that encodes a bioactivity or biomolecule.

I. The Sequence Listing

The Office Action asserts that the application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for failure to provide a Sequence Listing as set forth in 37 CFR 1.821(a)(1) and (a)(2). To comply with the requirements of the statute and the Notice To Comply With Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures received herein, Applicants submit herewith a Sequence Listing (1 page), a copy of the sequence information in computer readable form, and a Statement Under 37 C.F.R. § 1.821(f) and (g) that the enclosed Sequence Listing includes no new matter. Applicants request entry of the Sequence Listing in the application following the Abstract on page 79 and before the Drawings.

II. Claim Objections

Applicants traverse the objections to claims 11, 16 and 12 for alleged informalities. These claims have been canceled and replaced with new claims, thus rendering the rejection moot as to original claims 11, 16 and 12. With regard to claim 12, the Office Action alleges that "C12FDG should be spelled out. However, C12FDG is the name of a staining reagent. In new claim 30, C12FDG is clearly identified as a staining reagent. With regard to claim 16, the Office Action alleges that use of the term FACS introduces an informality. Accordingly, in new claim 35, FACS is identified as "fluorescence activated cell sorting." The Office Action further alleges that claim 12 is improperly dependent because it fails to further limit the subject matter of an earlier claim. Applicants submit that new claim 30, which contains the requirement of canceled claim 12, is properly dependent upon new independent claim 19. Therefore, Applicants

respectfully submit that any informalities in original claims 11, 16 and 12 are not contained in new claims 19-42.

III. The Rejection under 35 U.S.C. § 112, Second Paragraph

Applicants traverse the rejection of claims 1-18 for allegedly being indefinite under 35 U.S.C. § 112, Second Paragraph. With regard to the term "sample(s)" in claim 1, the Examiner alleges that the term is used to refer both to genomic DNA and to host cells. In new claim 19, which corresponds to original claim 1, the term "samples" is not used, thus rendering the rejection moot with regard to this issue.

With regard to the term "normalized" in claim 1, the Examiner alleges that the term is not defined by the claim and that the Specification does not provide a standard for ascertaining the requisite degree. Therefore, the Examiner asserts that for purposes of the Office Action, the term is understood to mean "DNA which has undergone the normalization procedure described on page 69, line 20 to page 70, line 6 (Office Action, page 4). However, the Specification also describes "normalization" of libraries at page 7, lines 18-23 and at page 24, line 11 to page 26, line 12. Thus, Applicants submit that the Specification provides a broader meaning for the term "normalized" than is proposed by the Examiner. However, with regard to the alleged indefiniteness of original claim 1, Applicants submit that the replacement of claim 1 with new claim 19, which does not require normalization of the library, renders the rejection moot.

With regard to the term "multispecific" in claim 1, the Examiner alleges that the term is not defined by the claim and that the Specification does not provide a standard for ascertaining the requisite degree. Therefore, the Examiner asserts that it is unclear what a multispecific expression library would be (Office Action, page 4). However, Applicants submit that the replacement of claim 1 with new claim 19, which does not require a "multispecific" expression library, renders the rejection moot.

With regard to original claim 11, the Examiner alleges that use of the term "comprising" introduces indefiniteness, apparently because the claim does not state what the substrate would contain in addition to C12FDG. However, Applicants submit that those of skill in the art are familiar with the convention that "comprising" claim language signals open claim format and would understand that a substrate containing the dye C12FDG could readily contain such additional entities as a chemical linker, and the like. Thus, Applicants submit that the phrase "comprises C12FDG" in new claim 30 is definite under 35 USC 112, Second Paragraph.

With regard to original claims 14 and 15, the Examiner alleges that the term "range introduces indefiniteness because a single temperature of about 70°C or a single time of about 30 minutes, respectively, is not a range" (Office Action, page 4). Applicants respectfully submit, however, that the term "about X" does limit a claim to a "single" value. However, to reduce the issues and expedite prosecution, in new claims 33 and 34, which correspond to original claims 14 and 15, the phrase "a range of about X" has been replaced by the phrase "about X," thus avoiding any indefiniteness that might have been introduced by the term "range" as used in the original claims.

With regard to original claim 18, the Examiner asserts the claim is "unclear as it refers to the method of claim 1 but does not make clear what the instant method is supposed to do" (Office Action, page 5). Applicants submit that claim 18 requires an additional method step in the process of claim 1 for identifying enzymes encoded by DNA. Hence original claim 18 properly depends from original claim 1.

By the present communication, however, original claims 1 and 18 have been canceled, thus rendering the rejection moot as to original claim 18. New claim 40, which parallels original claim 18 and depends from claim 19, adds two steps to the method of the claim from which it depends (i.e., "obtaining DNA from a clone identified in step c) that is positive for an enzymatic activity of interest and comparing the enzymatic activity

of a DNA expression product from the clone with that obtained from such a clone into whose DNA at least one mutation has been introduced, wherein a difference in enzymatic activity is indicative of the effect upon the enzymatic activity of interest caused by introduction of the at least one mutation"). As in all dependent claims, the additional requirements in dependent claim 40 are clearly required steps in Applicants' "method for identifying a bioactivity or biomolecule of interest." Accordingly, Applicants submit that new claim 40 is definite under 35 USC §112, Second Paragraph.

In view of the replacement of claims 1-18 with new claims 19-45 and the above arguments, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 USC §112, Second Paragraph.

IV. The Rejection under 35 U.S.C. § 112, First Paragraph

Applicants traverse the rejection of claim 2 for allegedly lacking enablement under 35 U.S.C. § 112, First Paragraph. In support of the rejection, the Examiner asserts:

> ... the specification, while being enabling for a method of screening DNA to identify lipases, esterases, glycosidases, proteases and monooxygenases through the use of bioactive fluorescent substrates does not reasonably provide enablement for the use of said screening method to identify glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases and acylases. ...Insufficient examples are provided of fluorescent substrates which can be used to assay enzymes besides lipases, esterases, glycosidases, proteases and monooxygenases. ...The relative skill of those in the art is low in determining which fluorescent substrates can be catalyzed by a particular enzyme and used as an indicator of enzymatic activity.

(Office Action, page 5). Applicants submit that those of skill in the art can practice the invention using the guidelines provided by the Specification without undue experimentation. Applicants disclose use of substrates that are fluorescent in the presence of the enzymatic activity of glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases,

transaminases, amidases and acylases (Specification, pages 34-39).°D The technique for using such substrates in practice of the invention methods does not differ substantially from that illustrated in the Examples wherein the enzymes of interest are different, e.g., lipases, esterases, glycosidases, etc).

In addition, Applicants disagree with the Examiner's assertion that the relative skill of those in the art is low regarding selection of substrates that can be catalyzed by a particular enzyme as an indicator of enzymatic activity. Applicants respectfully submit that, in fact, substrates that fluoresce in the presence of the enzymatic activity of glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases and acylases were well known in the art at the filing date of the present application. Especially well known at the filing date of the present application are substrates of acylases, amidases, transaminase, kinases, and phosphatases, as can be determined by a review of the relevant technical texts.

Therefore, Applicants request reconsideration and withdrawal of the rejection of claim 2 for alleged lack of enablement under 35 USC §112, First Paragraph.

V. The Rejection under 35 U.S.C. § 102(e)

The rejection of claims 1-18 under 35 U.S.C. § 102(e) for alleged anticipation by Thompson et al., (U. S. Patent No. 5,824,485, hereinafter "'Thompson") is respectfully traversed. Applicants' invention method for identifying bioactivity or biomolecule using high throughput screening, as defined by claim 19, distinguishes over the disclosure of Thompson by requiring screening of a library containing a plurality of clones obtained from more than one organism (i.e., a mixed population of either uncultured or isolated organisms). Generally, the clones of the library contain DNA obtained from a single organism within the mixed population. Thus, Applicants' method involves cloning of individual genes or groups of genes (e.g., pathways) obtained from an organism, into a host cell. The clones in Applicants' "natural"

library include host cells containing DNA (e.g., single genes or pathways) obtained from an organism that may encode one gene product or more than one gene product.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration (In re Spada, 15 USPQ 2d 1655 (Fed. Cir. 1990), In re Bond, 15 USPQ 2d 1566 (Fed. Cir., 1990).

By contrast to Applicants' invention method, the libraries described by Thompson are "combinatorial natural pathway expression library" containing "expression constructs prepared from genetic material obtained from one or more species of donor organisms in which genes present in the genetic material are operably associated with regulatory regions that drive[s] expression of the genes in an appropriate host organism" (Thompson, Col. 9, lines 27-32). In many cases, the clones do not each contain DNA from a single organism, but are synthetically produced, for example, by replacing one or more genes of a known pathway from organism X with one or more genes from the same pathway from organism Y in order to artificially create "new" pathways. Alternatively, one or more genes from pathway X may be replaced with one or more genes from pathway Y. In other examples provided by Thompson, a single host cell, which might contain a known gene A, from organism A, can be transformed with gene B from organism B and gene C from organism C, thereby producing a novel metabolic pathway. These novel pathways are synthetically produced and contain genes combined from various organisms. Thus, Thompson teaches a "combinatorial library" containing genomic DNA in which individual genes from different species can be concatenated in such a way as to produce a novel, non-naturally occurring pathway. However, Thompson is silent regarding preparation of a library of naturally occuring genes or gene pathways in which each clone may contain any type of DNA and wherein the DNA in each clone is obtained from an organism from a mixed population of organisms. Therefore, Thompson fails to teach each and every element of Applicants' method for high throughput screening as would be required to show anticipation under 35 USC§ 102(e).

Moreover, Applicants submit that Thompson also fails to suggest a method for high throughput screening of "natural" libraries under 35 USC § 103 to identify a bioactivity or a biomolecule of interest. In particular, Thompson fails to suggest that a library containing a plurality of clones obtained from more than one organism, but wherein each clone contains DNA from one organism could be used to discover such bioactivities as result from natural but previously unknown pathways.

In high throughput screening of complex environmental libraries to identify genes encoding bioactivities or biomolecules of interest, the rate limiting steps in discovery occur at both the DNA cloning level and at the screening level. For example, a complex environmental library may contain hundreds of different organisms, requiring the analysis of several million clones. Such a library can be screened with relative ease by using Applicants' method because each clone contains the DNA derived from one organism in the multispecies population.

In addition, Applicants' method enhances the discovery of bioactivities within a complex environmental library containing, for example, DNA from thousands of different organisms. Applicants disclose a method for screening anywhere from about 30 million to about 200 million clones per hour for a desired biological activity so that naturally occurring pathways expressing novel biomolecules can be identified without any requirement for a preselection or combinatorial rearrangement of genes of a known pathway producing a known biomolecule. Rather, the method of the invention allows identification of, in many cases, previously unknown, naturally occurring pathways and genes and the biomolecules produced therefrom.

Secondly, Applicants disclosed invention method can be used to screen such organisms as Actinomycetes, or Streptomyces on filamentous fungi and bacteria which have, at one stage of their life cycle, unicells or monocells (multinucleoid cells fragment to produce monocells) (Specification, page 56, line 6 to page 57, line 6) Typically, spores of myceliate organisms germinate to make substrate mycelia (during which phase antibiotics are potentially produced),

which then form arial mycelia. Arial mycelia eventually fragment to make more spores. Any filamentous bacteria or fungi which forms monocells during one stage of its life cycle can be screened for an activity of interest. Previously, this was not done because a branching network of multinucleoid (fungal like) cells forms with certain species. In one embodiment, the present invention presents a particular species, *Streptomyces venezuelae*, for screening utilizing a fluorescent analyzer which requires single cell detection. The method of the present invention allows one to perform high throughput screening of myceliates for production of, for example, novel small molecules and bioactives.

Streptomyces venezuelae, unlike most other *Streptomyces* species, has been shown to sporulate in liquid grown culture. In some media, it also fragments into single cells when the cultures reach the end of vegetative growth. Because the production of most secondary metabolites, including bioactive small molecules, occurs at the end of log growth, it is possible to screen for *Streptomyces venezuelae* fragmented cells that are producing bioactivities by a fluorescence analyzer, such as a FACS machine, given the natural fluorescence of some small molecules.

In one aspect of the present invention, any *Streptomyces* or *Actinomyces* species that can be manipulated to produce single cells or fragmented mycelia is screened for a characteristic of interest (see for example specification, pages 56-58). It is preferable to screen cells at the stage in their life cycle when they are producing small molecules for purposes of the present invention. Applicants submit that Thompson's disclosure of screening combinatorial libraries produced from known genes fails to suggest that the natural libraries required by claim 1, which can include such difficult organisms as *Streptomyces* or *Actinomyces*, could produce biomolecules in sufficient amounts to turn over a detectable amount of substrate.

The method of the invention requires a substrate which is able to enter the cell and maintain its presence within the cell for a period sufficient for analysis to occur. It has generally

been observed that introduction of the substrate into the cell across the cell membrane occurs without difficulty. It is preferable that once the substrate is in the cell it not "leak" back out before reacting with the biomolecule being sought to an extent sufficient to produce a detectable response. Retention of the substrate in the cell can be enhanced by a variety of techniques. In one disclosed by Applicants, the substrate compound is structurally modified by addition of a hydrophobic tail (e.g., see claim 31). Alternatively, preferred solvents, such as DMSO or glycerol, can be administered to coat the exterior of the cell. Also the substrate can be administered to the cells at reduced temperature, which has been observed to retard leakage of the substrate from the cell's interior. A broad spectrum of substrates can be used in practice of the invention method which are chosen based on the type of bioactivity sought. The substrate is selected to interact with the target biomolecule to produce a detectable response. Applicants submit that Thompson fails to suggest methods useful for causing a fluorescent molecule to enter cells in a library and remain within the cells for a period of time sufficient to conduct high throughput screening of large libraries of molecules.

For the reasons discussed above, Applicants submit that Thompson neither anticipates nor renders obvious the invention as defined by new claims 19-42. Accordingly, Applicants respectfully request that this rejection be withdrawn.

VI. The Rejection under 35 U.S.C. § 103(a)

A. Applicants respectfully traverse the rejection of claims 1 and 2 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson as applied in Section III above in view of Nadar et al. (U.S. Patent No. 5,173,187; hereinafter "Nadar"). Applicants respectfully disagree with the Examiner's assertion:

> It would have been obvious to one of ordinary skill in the art at the time the invention was made to screen an expression library, as taught by Thompson et al., for esterase activity using a fluorescent substrate, as taught by Nadar et al. One of ordinary skill in the art is motivated to do this for the benefit of identifying a clone of the expression library which expresses an esterase.

(Office Action, page 6.

As discussed above in Section III, Applicants' invention method for identifying an activity of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon), distinguishes over the disclosure of Thompson by screening of a library containing a plurality of clones derived from more than one organism.

The disclosure of Nadar fails to overcome these deficiencies in Thompson. Like Thompson, Nadar is completely silent regarding screening of a library containing a plurality of clones derived from more than one organism wherein each clone contains DNA derived from a single organism. Instead, Nadar discloses identification of species of bacteria in sludge. Accordingly, Applicants respectfully submit that the combined disclosures of Thompson and Nadar are not sufficient to establish the prima facie obviousness of Applicant's invention as defined by present claims 20 and 21 (which correspond to original claims 1 and 2).

B. Applicants respectfully traverse the rejection of claims 1 and 3 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson. As discussed above in Section III, Applicant's invention method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon), clearly distinguishes over the disclosure of Thompson. Applicant submits that new claim 23, which contains all the limitations of claim 19 from which it depends, further distinguishes over Thompson in that it contains the further requirement of original claim 3 that the prokaryotic library contains "at least about 2×10^6 clones." Therefore, Applicant submits that Thompson fails to teach or suggest Applicant's invention of new claim 23.

C. Applicants respectfully traverse the rejection of claims 1, 3, 10, 11 and 12 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson in view of Miao et al.

(*Biotechnology and Bioengineering* 42:708-715, 1993; hereinafter "Miao"). Applicant respectfully disagrees with the Examiner's assertion:

> It would be a reasonable expectation to be able to use FACS screening of an expression library, as shown by the teachings of Thompson et al., with the fluorescent β-galactosidase substrate C12FDG, as shown by the teachings of Miao et al., to isolate β-galactosidase expressing clones as the teachings of Miao et al. show using fluorescence flow cytometry analysis of *E. coli* using C12FDG. Additionally, C12FDG comprises a lipophilic tail.

(Office Action, pages 10-11).

The deficiencies of Thompson for disclosing or suggesting Applicants' invention method for identifying an bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon) are discussed in Section III above and are incorporated here by reference. Applicant respectfully submits that Miao fails to cure the deficiencies of Thompson. Miao's disclosure pertains to use of C12FDG as a fluorescent substrate in FACS screening of single bacterial cells of one species (i.e., E. coli). The focus of Miao's study is optimization of substrate concentration. However, like Thompson, Miao is completely silent regarding screening of a library containing a plurality of clones obtained from one or more organism wherein each clone contains DNA of a single organism. Accordingly, Applicant respectfully submits that the combined teachings of Thompson and Miao as alleged by the Examiner, including Miao's disclosure regarding C12FDG, are not sufficient to teach or suggest Applicant's invention of new dependent claims 22, 31, 32 and 33, which contain the requirements, respectively, of original claims 3, 10, 11 and 12.

D. Applicant respectfully traverses the rejection of claims 1 and 17 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson. As discussed above in Section III, Applicant's invention method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon), clearly distinguishes over the disclosure of Thompson. Applicant submits, therefore, that any disclosure

of Thompson pertaining to activities that the Examiner alleges are analogous to biopanning is not sufficient to render obvious Applicant's invention of new claim 39, which contains the additional requirement of original claim 17 that the expression library is biopanned prior to stable insertion of the substrate. Therefore, Applicant respectfully submits that Thompson fails to teach or suggest Applicant's invention of new claim 39.

E. Applicant respectfully traverses the rejection of claims 1 and 13-15 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson as applied above in view of Miao. With regard to Miao, the Examiner states:

> Miao et al. teach ("Abstract," page 708; "Single-Cell β-galactosidase activity by fluorescence flow cytometry using the fluorescent substrate C12FDG and growing E. coli cells at 37°C for a few minutes to an hour to allow permeation and reaction of the fluorescent substrate. Miao do not teach a method of screening an expression library or heating cells at 70°C. ...It would have been obvious to one of ordinary skill in the art to heat the samples at a temperature required for activity of the enzymes being screened.

(Office Action, page 12).

As discussed above in Paragraph B of this section, Applicant's invention method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon), clearly distinguishes over the disclosure of Thompson. The disclosure of Miao does not overcome these deficiencies of the primary reference with regard to the identifying a bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19. Miao is completely silent regarding screening of a library containing a plurality of clones obtained from one or more organism wherein each clone contains DNA from one organism in the multispecies population. Accordingly, Applicant respectfully submits that the combined teachings of Thompson and Miao, including Miao's disclosure regarding heating of the cells ,as alleged by the Examiner are not sufficient to disclose or suggest Applicant's invention as defined by new claims 33 and 34, which correspond to original claims 13 and 15.

Therefore, Applicants submit that new claims 19-42 are not obvious under 35 U.S.C.§ 103 over the disclosure of Thompson or the disclosure of Thompson in combination with that of Nadar or Miao.

In view of the above amendments and remarks, reconsideration and favorable action on new claims 19-45 are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures:

Paper of copy of the Sequence Listing
Copy of the sequence information in computer readable form
Statement Under 37 C.F.R. § 1.821(f) and (g)